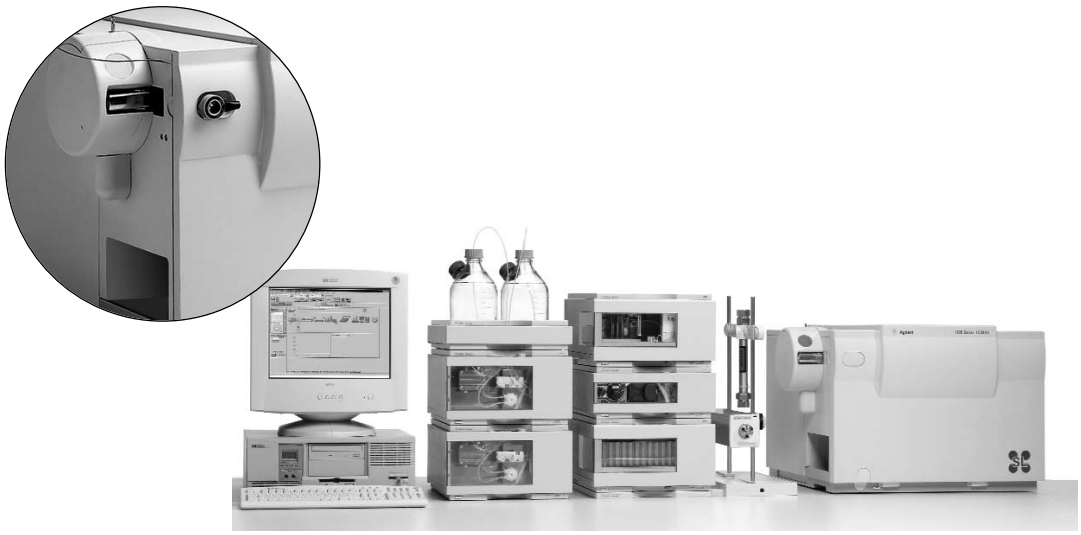
Compound purification using the Agilent 1100 Series purification system with a mass-selective detector

Application

Udo Huber



Abstract

Preparative HPLC is one of the major techniques for purification of compounds in drug discovery. While time- and peak-based fraction collection are established methods, the availability of easy-to-use and non-expensive mass-selective detectors (MSD) nowadays enhances the possibilities of compound purification. In this Application Note we show the advantages of mass-based fraction collection and how an MSD in the Agilent 1100 Series Purification system[1,2] can enhance and simplify time- and peak-based fraction collection.

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Introduction

When purifying samples using preparative HPLC two aspects are important. First, the number of collected fractions should be as low as possible – the optimum is one fraction per sample containing the target compound. Collecting too many fractions leads to tedious re-analysis to identify the desired compound. Second, very often the "baseline", i.e. everything between the fractions, should also be collected, for example, to recover starting material that has not been converted or in case something important was not collected.

In this Application Note we show three strategies for fraction collection leading to either many or only a single fraction, with complete or low recovery of the injected sample. However, in all three cases, there is no need for fraction re-analysis to identify the desired target compound. This is achieved by connecting an 1100 Series mass selective detector[3] to the Agilent 1100 Series purification system.

Note:
To avoid losing those parts of the injected sample that are not collected as fractions, the Agilent 1100 Series purification system offers so-called recovery locations. These locations are assigned to a specific sample and are used to collect everything that is not a fraction.

Equipment

The system used included:

- Two Agilent 1100 Series preparative pumps
- Agilent 1100 Series preparative autosampler
- Agilent 1100 Series column organizer
- Agilent 1100 Series diode array detector
- Agilent 1100 Series fraction collector PS
- Agilent 1100 Series mass-selective detector (MSD)
- Agilent 1100 Series isocratic pump (as make-up pump)
- Agilent active splitter

The system was controlled using the Agilent ChemStation (rev. A.09.01) and the Purification/ High Throughput software (rev. A.01.01).

Results and Discussion

In this Application Note we show the following three purification strategies:

1. Time-based fraction collection monitored with an MSD
2. Peak-based fraction collection monitored with an MSD
3. Mass-based fraction collection

1. Time-based fraction collection monitored with the MSD

Fractions were collected using time-based fraction collection, which guarantees that almost nothing of the injected sample is lost. The strategy was further optimized by using the fraction collection features *shortest way* and *continuos flow*. In the collection mode *continuos flow* the fraction collection valve does not switch to the waste position when the

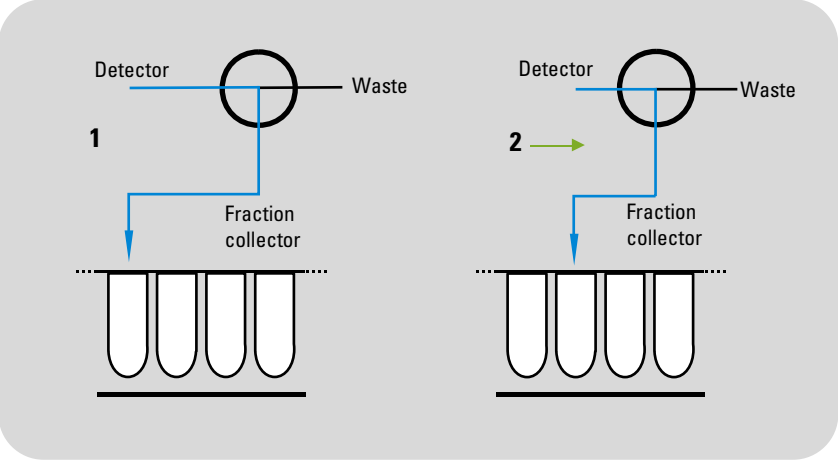

Figure 1
***Continuos flow* feature**

collection needle moves from one fraction position to the next (figure 1). This feature is only available when using well-plates as fraction containers to avoid eluent spilling

The filling order *shortest way* fills the fraction containers in a meandering sequence, which ensures that the needle always uses the shortest path when moving between fraction positions (figure 2).

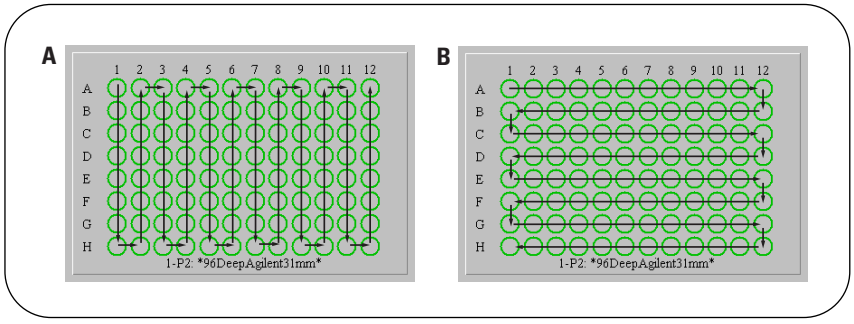

Figure 2
A. *Shortest way* by columm, B. *Shortest way* by row

Since the sample was dissolved in DMSO the method was set-up to make sure the DMSO elutes before any compound of interest. Therefore, water/acetonitrile 90:10 was pumped through the column for two minutes before the gradient was started. To avoid contamination of the MSD with DMSO the stream selection valve was switched to the waste position for the first 2 minutes of the run. Fractions were collected based on time, collecting 80 fractions between 2 and 7.5 min and resulting in the chromatograms shown in figure 3. The vertical lines show the start tick-marks of the collected fractions.

Columns	ZORBAX SB-C18 21.2 × 50 mm, 5 µm
Mobile phases:	A= water, B= acetonitrile
Gradient:	at 0 min 10 % B at 2 min 10 % B at 6 min 90 % B at 7 min 90 % B at 8 min 10 % B
Stop time:	8 min
Post time:	2 min
Flow rate:	25 mL/min
Injection:	100 µL
Column temp.:	ambient
UV detector:	DAD: 220/16 nm (ref. 360/60 nm), preparative flow cell (0.3-mm pathlength)
MSD	
Make-up flow:	1 mL/min
Make-up solvent:	water/acetonitrile 25:75 + 0.1 % HOAc
Ionization mode:	API-ES positive
Scan-range m/z:	150 - 500 starting at 2 min
Fragmentor:	70 volt
Drying gas flow:	13 L/min
Nebulizer pressure:	55 psig
Drying gas temp.:	350 °C
Cap. voltage:	3000 V

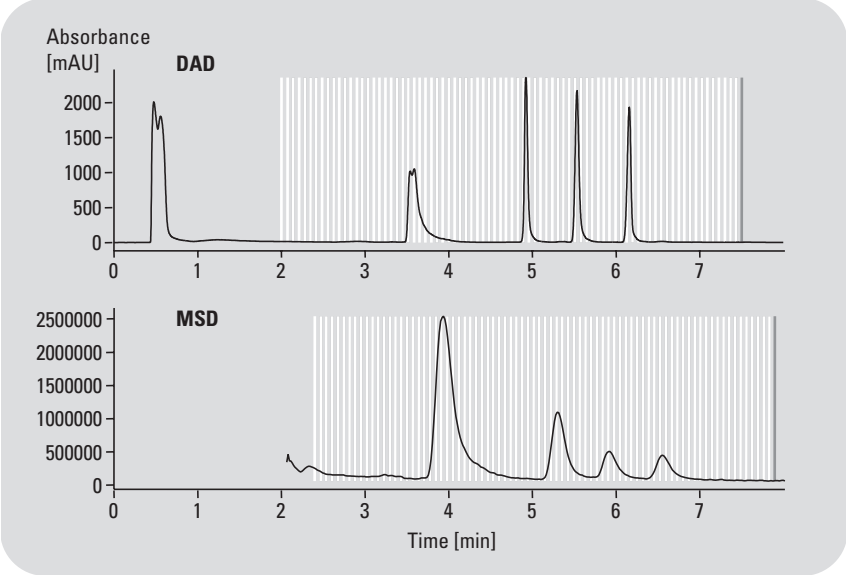

Figure 3
Result of time-based fraction collection

To identify the fractions containing the desired compound (target mass 241 amu) the extracted ion chromatogram (EIC) of the mass 242 ($[M+H]^+$) was overlaid with the total ion chromatogram (TIC). Figure 4 shows the fractions containing the target mass in the well-plate positions B-02 to B-06. The overall number of collected fractions was 80 and the loss of injected sample after the initial two minutes was 0 %.

2. Peak-based fraction collection monitored with an MSD

Using the second strategy, the fractions were collected based on the peaks in the UV signal with a threshold setting of 50 mAU. This ensures that the main peaks of the sample are collected but the number of collected fractions is dramatically reduced compared to time-based fraction collection. The resulting chromatograms, measured with the same method as described in figure 3, are shown in figure 5.

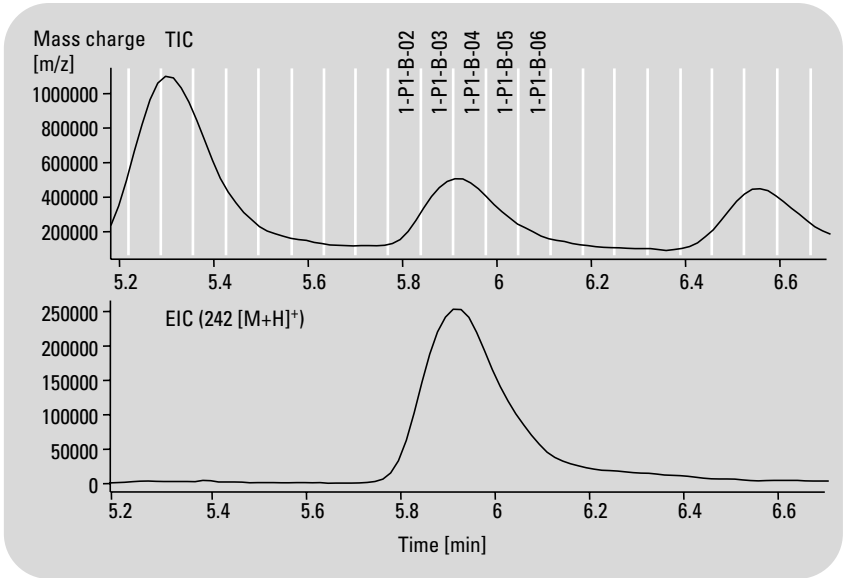

Figure 4
Overlaid TIC and EIC to identify the desired fractions

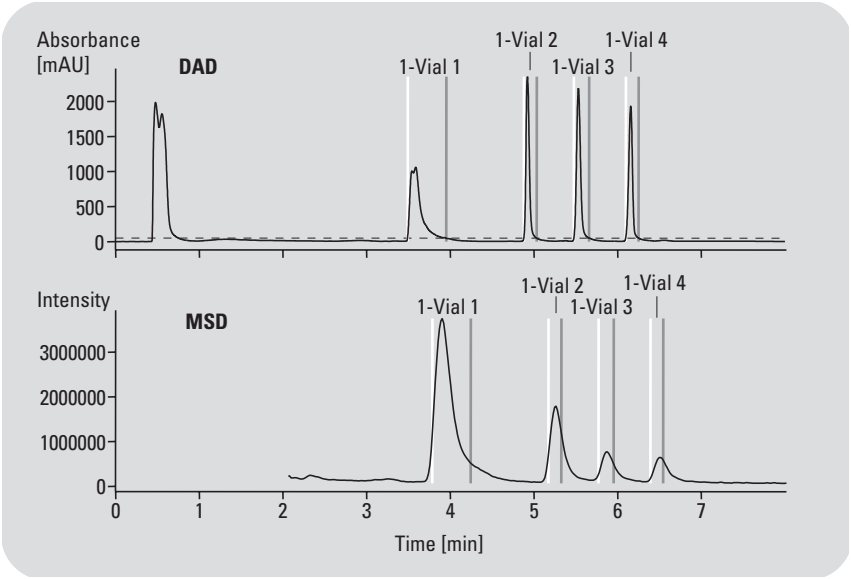

Figure 5
Result of peak-based fraction collection

To identify the fractions containing the desired compound the EIC of the mass 242 ($[M+H]^+$) was again overlaid with the TIC (figure 6).

Figure 6 shows that the fraction containing the target mass is in vial number 3. The number of collected fractions decreased to 4, however, the loss of sample after the initial two minutes increased to 80 %.

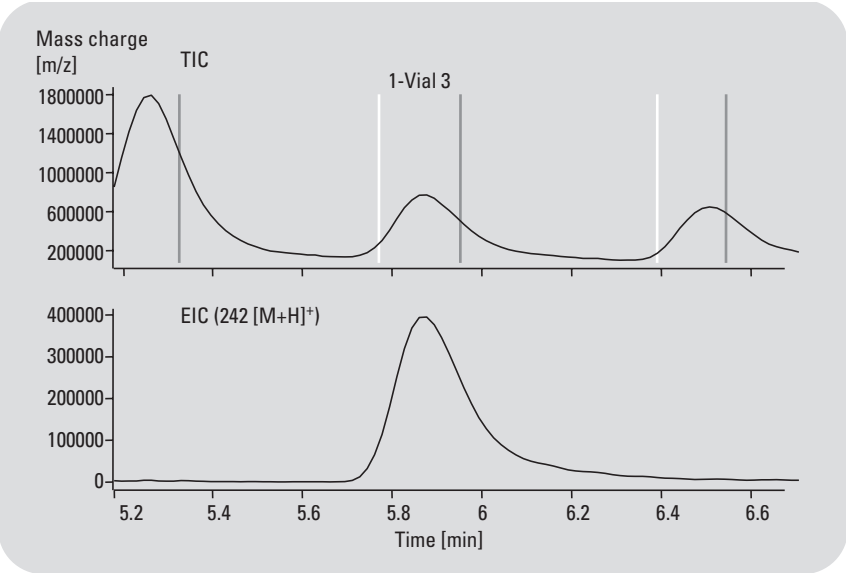

Figure 6
Overlaid TIC and EIC to identify the desired fractions

3. Mass-based fraction collection

When using mass-based fraction collection a fraction is only triggered when a peak in the MSD contains the desired target mass and the EIC of this target mass exceeds the specified threshold. This ensures that in most cases only a single fraction per sample run containing the desired compound is collected. The disadvantage is that most of the injected sample is not recovered. The result of the mass-based fraction collection is shown in figure 7.

As figure 7 shows, only one fraction containing the target mass was collected in vial number 1, therefore the number of collected fractions decreased to 1, however the sample loss increased to 95 %.

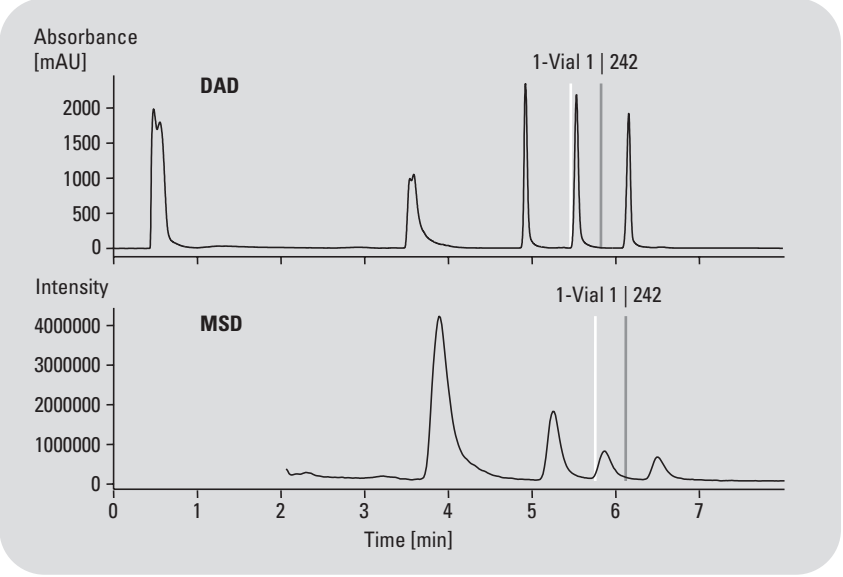

Figure 7
Result of mass-based fraction collection

Conclusion

In this Application Note we showed three strategies for sample purification with an MSD connected to the Agilent 1100 Series purification system. For time-based fraction collection, monitored with the MSD, the number of collected fractions is very high, however, almost the entire injected sample is recovered. Peak-based fraction collection monitored with an MSD decreases the number of fractions dramatically, but only the main peaks of the sample are collected. Small peaks and the entire baseline are not recovered. Using mass-based fraction collection, the number of collected fractions is reduced to a single fraction in most cases, however most of the sample is not recovered (figure 8).

While an MSD is not required for time- or peak-based fraction collection, it offers the advantage that tedious re-analysis of fractions to determine the ones containing the target compound is unnecessary. The desired fractions can easily be identified by overlaying the TIC with the EIC of the target mass.

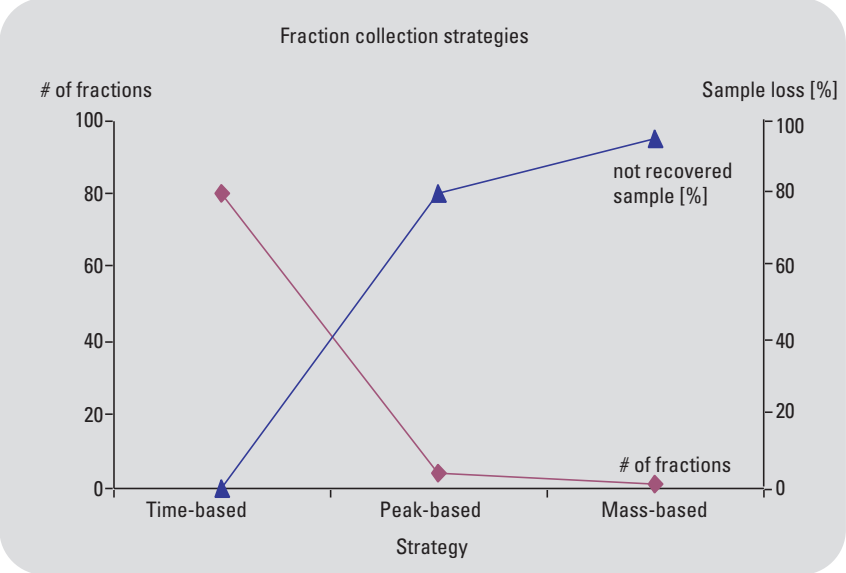

Figure 8
Number of collected fractions and percentage of not recovered sample for different fraction collection strategies

References

1.
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